BIOFUMIGANT COMPOUNDS RELEASED BY FIELD PENNYCRESS (*Thlaspi arvense*) SEEDMEAL[1]

STEVEN F. VAUGHN,* TERRY A. ISBELL, DAVID WEISLEDER,
and MARK A. BERHOW

New Crops and Processing Technology Research
USDA, ARS, National Center for Agricultural Utilization Research
1815 N. University St., Peoria, Illinois 61604, USA

Abstract—Defatted field pennycress (*Thlaspi arvense* L.) seedmeal was found to completely inhibit seedling germination/emergence when added to a sandy loam soil containing wheat (*Triticum aestivum* L.) and arugula [*Eruca vesicaria* (L.) Cav. subsp. *sativa* (Mill.) Thell.] seeds at levels of 1.0% w/w or higher. Covering the pots with Petri dishes containing the soil-seedmeal mixture decreased germination of both species at the lowest application rate (0.5% w/w), suggesting that the some of the phytotoxins were volatile. CH_2Cl_2, MeOH, and water extracts of the wetted seedmeal were bioassayed against wheat and sicklepod (*Senna obtusifolia* (L.) H. S. Irwin & Barneby) radicle elongation. Only the CH_2Cl_2 extract was strongly inhibitory to both species. Fractionation of the CH_2Cl_2 extract yielded two major phytotoxins, identified by gas chromatography–mass spectrometry and NMR as 2-propen-1-yl (allyl) isothiocyanate (AITC) and allyl thiocyanate (ATC), which constituted 80.9 and 18.8%, respectively, of the active fraction. When seeds of wheat, arugula and sicklepod were exposed to volatilized AITC and ATC, germination of all three species was completely inhibited by both compounds at concentrations of 5 ppm or less. In field studies, where seedmeal was applied at 0.50, 1.25, and 2.50 kg/m^2 and tarped with black plastic mulch, all of the treatments significantly reduced dry weight of bioassay plants compared to the tarped control, with the highest seedmeal rate decreasing dry matter to less than 10% of the control 30 d after seedmeal application. Field pennycress seedmeal appears to offer excellent

---

* To whom correspondence should be addressed. E-mail: vaughnsf@ncaur.usda.gov

[1] Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

167

potential as a biofumigant for high-value horticultural crops for both conventional and organic growers.

INTRODUCTION

Biofumigation is the exploitation of plant biochemicals with pesticidal properties that may serve as ecologically-friendly alternatives to chemical fumigants such as methyl bromide (Angus et al., 1995). Additionally, organic growers lack most of the soil fumigation/sterilant options available to conventional growers. Several plant families, especially the Brassicaceae or mustard family, have been extensively studied as green manure crops, as mustards have been shown to suppress weeds (Grossman, 1993; Boydston and Hang, 1995; Brown and Morra, 1995; Vaughn and Boydston, 1997; Al-Khatib et al., 1997; Boydston and Vaughn, 2002), soil pathogens (Papavizas, 1966; Lewis and Papavizas, 1971; Papavizas and Lewis, 1971; Ramirez-Villapudua and Munnecke, 1988; Muelchen et al., 1990; Vaughn et al., 1993; Mayton et al., 1996; Brown and Morra, 1997; Williams-Woodward et al., 1997; Olivier et al., 1998; Sarwar et al., 1998), nematodes (Mojtahedi et al., 1991, 1993; Donkin et al., 1995), and insects (Brown et al., 1991) when plant tissues were incorporated into the soil. Mustards are characterized by the presence of glucosinolates, a class of glucose and sulfur-containing organic anions (Figure 1), whose biologically-active degradation products are produced when plant vacuoles are ruptured and the glucosinolates present in vacuoles are hydrolyzed by the enzyme myrosinase (β-thioglucosidase glucohydrolase; EC 3.2.3.1) (VanEtten and Tookey, 1983). These products include substituted isothiocyanates, thiocyanates, nitriles, and oxazolidinethiones that vary depending on the side-chain substitution, pH, and iron concentration (Cole, 1976; Fenwick et al., 1983; Uda et al., 1986; Chew, 1988). Many of these hydrolysis products have pesticidal properties (Vaughn, 1999). Seedmeals from glucosinolate-containing plants have also been identified as potential soil amendments for weed and insect control (Brown and Morra, 1995; Vaughn et al., 1996; Walker, 1996; Elberson et al., 1997; Vaughn and Berhow, 1999). The wide range of glucosinolates found in the Brassicaceae provides the opportunity to select those species that have the highest biofumigation potential (Kirkegaard and Sarwar, 1999; Warton et al., 2001).

Field pennycress (*Thlaspi arvense* L.) is a weedy annual/winter annual species of the Brassicaceae that is a native of Europe but has a wide distribution throughout temperate North America (Rollins, 1993). Field pennycress is considered a major agricultural weed that competes with crops causing significant yield losses (Holm et al., 1997). The plant produces an unpleasant, garlic-like odor that has caused it to be widely known as stinkweed (Mitich, 1996). Each plant may produce
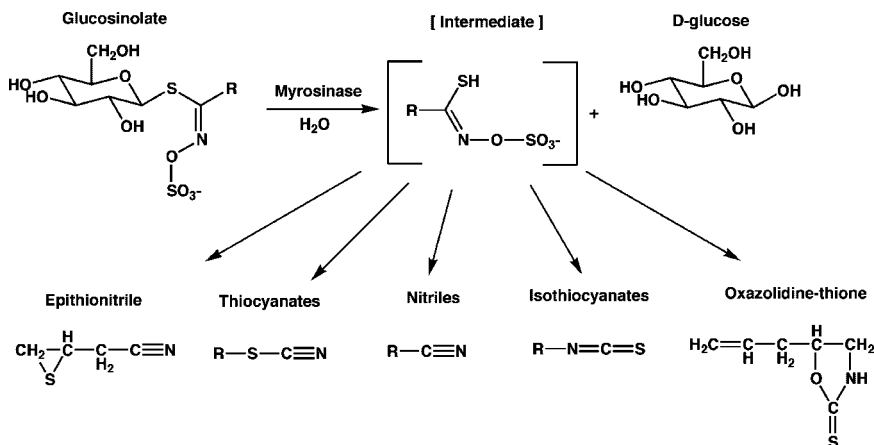
FIG. 1. The general structure of glucosinolates and their enzymatic degradation products.
Adapted from Rask et al. (2000).

up to 15,000 seeds, and fields heavily infested with field pennycress yield up to
1345 kg seed/ha (Best and McIntyre, 1975). Field pennycress seed contains the
glucosinolate sinigrin (Daxenbichler et al., 1991). Sinigrin can be hydrolyzed by
endogenous myrosinase to form principally allyl isothiocyanate (AITC), a potent
biocide that may be involved in allelopathy (Vaughn and Berhow, 1999), with al-
lyl nitrile, allyl thiocyanate (ATC), and epithiopropylnitrile also being formed at
other pH values or in the presence of reducing compounds (Cole, 1976; Springett
and Adams, 1989). However, Lüthy, and Benn (1977) found that field pennycress
seed flour extracts converted radiolabeled sinigrin into ATC without any AITC
being formed. While AITC has been shown to be an effective biocide against a
wide range of organisms, relatively little data have been published concerning
the biocidal activity of ATC (Shofran et al., 1998; Tsao et al., 2002). Our inter-
est in using glucosinolate-containing seedmeals as biofumigants prompted us to
further investigate the use of field pennycress seedmeal as a soil amendment for
weed suppression in high-value, low-acreage crops, and if active, to identify those
compounds responsible for phytotoxicity.

METHODS AND MATERIALS

Seedmeal Bioassays. Field pennycress seeds were harvested on June 30, 2003
from a native population present in a limited-tillage soybean field near Hanna
City, IL, USA Seeds were dried at room temperature for at least 30 d before use
in bioassays. Seeds were ground in coffee grinder and defatted with hexane for
24 hr in a Soxhlet apparatus. The dry seedmeal was stable for an extended period

($>$30 d at room temperature), but was used within 2 d of being defatted in all experiments. Seedmeal was thoroughly mixed with soil (Onarga sandy loam; Typic Argiudoll) at rates of 0.5, 1.0, and 5.0% (w/w). The seedmeal-soil mixture was added to 200-ml cups, and 10 seeds of wheat (*Triticum aestivum* L.) and arugula [*Eruca vesicaria* (L.) Cav. subsp. *sativa* (Mill.) Thell.] seeds (both of which exhibit nearly 100% germination) were added and covered with approximately 3 cm of the soil-seedmeal mixture. Nonamended soil was used as the control. Each cup received 10 ml of a solution containing 1g/l thiabendazole (excessive saprophytic fungal growth occurred at the higher seedmeal rates without this fungicide that we thought might influence the results), and then additional water was added to bring the soil to field capacity. Half of the treatments were covered with plastic Petri dishes for the initial 72 hr to prevent loss of potential volatile compounds, similar to what would be done with tarping in the field. All cups were placed in a growth chamber set at a 16 hr, 25°C d/8 hr, 20°C night regime. Emerged seedlings were counted after 14 d, and the above ground tissue of the emerged seedlings were counted. Each treatment was replicated five times in a completely randomized design, and the experiment was repeated. Data were subjected to analysis of variance (SAS, Cary, North Carolina).

Extract Preparation. One hundred grams of defatted seedmeal were wetted with 100 ml ddH$_2$O and allowed to sit in a beaker covered with aluminum foil for 4 hr. The wet seedmeal was extracted with three successive 250 ml aliquots of CH$_2$Cl$_2$. The CH$_2$Cl$_2$-extracted seedmeal was dried in a fume hood at room temperature to remove residual solvent, and then extracted $\times$3 with 250 ml aliquots of MeOH. Extracts from both solvents were pooled and concentrated by rotoevaporation. A water extract was obtained by soaking the solvent-extracted seedmeal in 250 ml of distilled water overnight in a refrigerator at 2°C, after which the marc was washed with two additional 250 ml aliquots, and the extracts lyophilized. The crude CH$_2$Cl$_2$ extract subsequently found to be active in the bioassays was separated on a lipophilic Sephadex LH-20 (Supelco, Inc., Bellefonte, PA) column into three separate fractions using 100% CHCl$_3$; 50% CHCl$_3$/50% MeOH; and 100% MeOH as solvents.

Seedling Radicle Elongation Bioassay. Wheat and sicklepod (*Senna obtusifolia* (L.) H. S. Irwin & Barneby) seeds were surface sterilized with 0.5% commercial chlorine bleach for 15 min, after which they were rinsed with and then soaked in sterile distilled water for 2 hr. Seeds were wrapped in sterile paper towels saturated with water and incubated overnight in darkness at 25°C. All crude and column extracts were assayed by adding extracts to which solvents had been completely evaporated to autoclaved water agar (1.0% w/v containing 500 mg/l chloramphenicol) at the rate of 1 mg extract/ml agar (this concentration is generally effective at identifying active fractions), after the agar had cooled to $\sim$40°C. Fifteen ml of the agar-extract mixtures were placed in 9.0-cm plastic Petri dishes, and five germinated seedlings were placed on the agar in the Petri dishes. The dishes

were incubated in darkness at 25°C on 45° slants for 24–48 hr, then evaluated for inhibition of radicle growth.

Chromatography and Spectroscopy. Gas chromatography–mass spectrometry (GC–MS) was performed using an Hewlett-Packard (HP) 6890 GC system attached to an HP 5972A Mass Selective Detector. Quantitation of compounds was performed on an HP 5890 gas chrmoatograph equipped with a flame ionization detector. Columns used in both systems were fused silica HP-5MS capillaries (0.25-μm-film thickness, 30 m $\times$ 0.25 mm ID). The GC operating parameters were as follows: splitless injection mode; temperature programmed from 40° to 315°C at 5°C/min with a 20-min initial temperature hold; He carrier gas flow rate at 1.1 ml/min, with the injector temperature set at 100°C, as ATC has been reported to decompose to AITC at higher injection temperatures (Lüthy and Benn, 1977). Spectra were compared with known standards or by computer with the Wiley/NBS Mass Spectral Registry (McLafferty and Stauffer, 1989). Proton and carbon-13 spectra were obtained on a Bruker Avance 500 spectrometer (Billerica, MA, USA) equipped with a 5-mm inverse broadband Z-gradient probe (^{1}H NMR, 500 MHz, ^{13}C, 125 MHz). The software used to obtain the NMR data was ICON 3.5. The solvent was deuterated chloroform.

Effect of Volatilized AITC and ATC on Seed Germination. AITC and ATC, the active compounds identified in the extract, were tested for inhibition of seed germination of wheat, annual ryegrass (*Lolium multiflorum* Lam.), sicklepod, arugula, and velvetleaf (*Abutilon theophrasti* Medicus). Due to the difficulties involved in isolating and purifying large amounts of the compounds from seedmeal extracts, synthetic AITC and ATC were used in these tests. ATC was synthesized according to the method of Slater (1992) while AITC was purchased from Sigma. Ten seeds of each species were placed into 9-cm Petri dishes on Whatman No. 1 filter paper disks saturated with sterile distilled water. The dishes were then placed into 2.4 l glass desiccators containing a filter paper disk onto which AITC or ATC were added (neat) on a volume compound-headspace volume basis at rates of 0.0, 0.1, 0.5, 1.0, 5.0, 10.0, and 20.0 ppm. Flasks were placed in darkness in a growth chamber at 25°C for 4 d, after which germination was scored. Each treatment consisted of three replicates per species and the experiment was repeated.

Isolation and Quantification of Glucosinolates from Seedmeal. For quantitation of seedmeal glucosinolates, a modification of a high-performance liquid chromatography (HPLC) method developed by Betz and Fox (1994) was used. Replicates of defatted seedmeal (5.0 g) were added to 200 ml boiling 70% (v/v) MeOH with stirring for 15 min, cooled, and then filtered through Whatman No. 2 filter paper. The marc was washed twice with 50 ml aliquots of 70% MeOH. The resulting extract was concentrated to 5–10 ml by rotoevaporation, and diluted to 25 ml to form a working solution. The extract was run on a Finnegan Thermoquest P4000 HPLC system (San Jose, CA) using a C$_{18}$ column (250 $\times$ 4.6 mm^2;

RP-18, 5; Licrosorb, Alltech, Deerfield, IL). Peaks were detected with a Finnegan Thermoquest P6000 photodiode array detector set at 237 nm. The initial mobile phase conditions were 12% methanol/88% aqueous 0.005M THS at a flow rate of 1 ml/min. The binary gradient was developed to 70% methanol/30% aqueous 0.005 M THS for 20 min, and held at these conditions for an additional 15 min. Quantitation was determined from a standard curve prepared from pure sinigrin (Sigma, St. Louis, MO). Each treatment was run in triplicate and the experiment was repeated.

Field Studies. Seedmeal was tested for its ability to suppress plant germination and growth when applied to tarped field plots. Experiments were conducted in a randomized complete block design with four replications (each replicate measured 0.75 m by 1.5 m) on a field site soil which was an Orthents complex containing a silt loam surface of 30–40 cm underlaid with a silt clay loam. The site was conventionally tilled 1 wk prior to experiments. In addition to native weed seeds present in the soil, each plot was spiked prior to seedmeal incorporation with 75 g of a bioassay seed mixture containing 1:1:1 amounts of annual ryegrass, white mustard (*Sinapis alba* L.), and sicklepod seeds. Defatted seedmeal prepared as previously described was applied to test plots at rates of 0.5, 1.0, and 2.5 kg/m^2 by sprinkling the seedmeal evenly over the test plots and incorporating the seedmeal into the soil with pitchforks to a depth of approximately 10 cm. The plots were then watered (approximately 5 l per plot) thoroughly, and covered with polyethylene plastic sheets (0.1-mm thickness, 0.91 m by 2 m; Sunbelt Plastics, Minneapolis, MN) that were covered with soil at the edges to prevent loss of volatiles. Controls consisted of untarped plots and tarped plots without seedmeal. Tarps were removed after 7 d, and all plots were watered as needed. After 30 d, the plant biomass of the plots was determined by digging up and washing all of the plants after which they were placed in a 50°C drying oven for 48 hr before being weighed. Data were subjected to analysis of variance (SAS, Cary, NC). Dry weight mean separation was performed using the Student–Newman–Keuls multiple range test ($P < 0.1$).

RESULTS AND DISCUSSION

Bioassays. After 14 d, field pennycress seedmeal added to soil completely inhibited germination of wheat and arugula at rates of 1.0% or higher (Table 1). Covering the cups increased inhibition at the 0.5% rate over uncovered meal at the same rate, suggesting that some or all of the phytotoxins were volatile. Indeed, when the covers were removed after 72 hr, all of the cups possessed a distinctive garlic-like odor regardless of application rate, which could also be detected initially from the seedmeal after watering. At the 5% rate, this odor was still detectable after 3 d from uncovered cups as well as covered ones.

Identification of the Phytotoxins. The crude CH$_2$Cl$_2$ extract strongly inhibited sicklepod and wheat radicle elongation, while the MeOH and water extracts had no

TABLE 1. INHIBITION OF WHEAT AND ARUGULA GERMINATION BY FIELD PENNYCRESS SEEDMEAL

Species	Germination (% of control)[a]					
	0.5% covered	0.5% uncovered	1.0% covered	1.0% uncovered	5.0% covered	5.0% uncovered
Wheat	15 ± 2.9b	40 ± 4.1c	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a
Arugula	32 ± 3.2b	73 ± 4.0c	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a

[a]Mean ± SE values within a row followed by the same letter were not significantly different by the Student–Newman–Keuls multiple range test ($P < 0.1$).

effect. Fractionation of the crude CH_2Cl_2 extract on a Sephadex LH-20 column yielded one fraction (100% $CHCl_3$) that was inhibitory to radicle elongation in bioassays when tested at 1 mg extract/ml agar. This fraction contained two major peaks as determined by GC–FID. The compounds were identified by GC–MS spectra and by NMR analysis as AITC and ATC. Prominent diagnostic mass spectral ions and their relative intensities for ATC are as follows: EI–MS [m/z (%)]: 99 (M^+, 91), 86 (6), 84 (10), 72 (35), 58(12), 41 (100). Prominent diagnostic mass spectral ions and their relative intensities for AITC are as follows: EI–MS [m/z (%)]: 99 (M^+, 100), 98 (9), 72 (28), 71 (8), 41 (47). The AITC and ATC peaks accounted for 80.9 and 19.1%, respectively, of the active fraction as quantitated by GC–FID. ^{1}H NMR and ^{13}C spectra for ATC are as follows: ^{1}H NMR δ ($CDCl_3$): 5.86 (1H, m) CH=; 5.32 (1H, d, $J = 16.8$ Hz) CH =; 5.28 (1H, d, $J = 10$ Hz); 3.49 (2H, d, $J = 7.3$ Hz) CH_2. ^{13}C NMR δ ($CDCl_3$): 130.5 (C=); 121.4 (CH_2=); 111.7 (C); 36.7 (CH_2). ^{1}H NMR and ^{13}C spectra for AITC are as follows: ^{1}H NMR δ 5.78 (1H, m) CH=; 5.33 (1H, d, $J = 16.9$ Hz) CH =; 5.22 (1H, d, $J = 10.3$ Hz) CH =; 4.08 (2H, m) CH_2. ^{13}C NMR δ ($CDCl_3$): 132 (C); 130.3 (CH=); 117.7 (CH_2=); 47.1 (CH_2). In the proton NMR spectrum of ATC, the aliphatic methylene protons are observed at δ 3.49, in the AITC spectrum the methylene protons resonate at δ 4.08. Differences in the ^{13}C spectra for the two compounds are striking. The resonance for the nonprotonated carbon in ATC is observed at δ 111.7. In AITC, the nonprotonated carbon is observed as a small, broad signal at δ 132.0. The changes in aliphatic methylene resonances also demonstrate the difference between the two compounds.

Intact Glucosinolate Analysis. Analysis of intact glucosinolates from defatted seedmeal by HPLC found only one major glucosinolate peak, tentatively identified by retention time as sinigrin. LC–MS analysis of the peak confirmed the mass of the unknown to be 358, affirming that the compound was indeed sinigrin. Total amounts of sinigrin contained in the seedmeal were found to be 39.7 ± 3.8 mg sinigrin/g defatted seedmeal.

Inhibition of Seed Germination by AITC and ATC. Both AITC and ATC were inhibitory to seed germination, with 5 ppm levels of both compounds completely

TABLE 2. MINIMUM INHIBITORY CONCENTRATIONS OF AITC AND
ATC

	Concentration (ppm)				
Volatile	Wheat	Ryegrass	Arugula	Sicklepod	Velvetleaf
AITC	1	0.5	5	5	1
ATC	1	1	5	1	0.5

inhibiting the germination of the five species tested (Table 2). The lowest level tested (0.1 ppm) did not inhibit the germination of any of the species, although annual ryegrass seed germination was completely inhibited by both compounds at 0.5 ppm levels. Interestingly, arugula, the only bioassay species to possess the glucosinolate-myrosinase system, was the most tolerant of the five species to both compounds. Although thiocyanates have been shown to have activity against plants, they are generally thought to be less active than their respective isothiocyanates (Vaughn, 1999). However, Shofren et al. (1998) found that ATC had similar inhibitory activity to AITC against several bacteria, while Tsao et al. (2002) found that the LC_{50} (concentration required to kill 50% of the population) values of ATC as a fumigant against house fly (*Musca domestica* L.) and lesser grain borer (*Rhyzopertha dominica* Fabricius) adults was lower than AITC.

Field Studies. At all three rates tested, field pennycress seedmeal reduced plant biomass as compared to both the tarped and untarped controls (Figure 2). At the highest rate of incorporated seedmeal, biomass was reduced to less than 9% of the tarped control and less than 8% of the untarped control. All of the treatments exhibited visual reductions in seed germination compared to controls, and in the two highest treatment levels the ground remained completely bare in the center of each plot at the time of harvest. This seems to indicate that volatile compounds were primarily responsible for the inhibition, as leakage of volatiles near the edges of the tarps would decrease their concentrations compared to the centers. At the $0.5\,kg/m^2$ rate, there was some fungal growth found upon tarp removal near the plot edges that was lacking at the higher rates, although visible evidence of this fungi dissapeared within several days after removal of the tarps. Tarping alone appeared to promote rapid seed germination, possibly due to increased soil temperatures under the tarps, but many of the seedlings that had germinated either died after tarp removal or took longer to recover.

Using glucosinolate-containing plant material and processed seedmeals offer excellent potential in controlling weeds and soilborne pests, whether for organic or conventional growers. Because the bioactive degradation products dissapate rapidly, they pose less environmental risk than compounds that persist for greater periods of time. Field pennycress seedmeal offers potential as a biofumigant if the
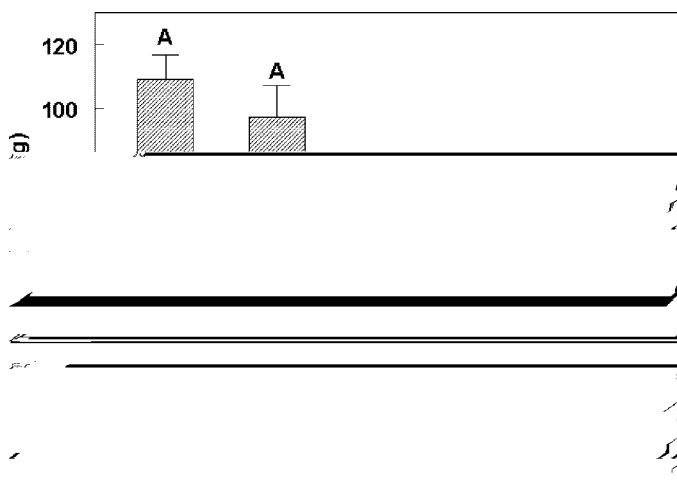
FIG. 2. Weed biomass in field plots 30 d after seedmeal application. Bars represent 1 S. E. Treatments with the same letter are not significantly different by the Student–Newman–Keuls multiple range test ($P < 0.1$).

agronomic obstacles associated with growing the plant as a crop are solved. Additionally, unlike synthetic fumigants, field pennycress seedmeal also is an excellent organic fertilizer, containing approximately 4% nitrogen.

REFERENCES

AL-KHATIB, K., LIBBEY, C., and BOYDSTON, R. 1997. Weed suppression with *Brassica* green manure crops in green pea. *Weed Sci*. 45:439–445.

ANGUS, J. F., GARDNER, P. A., KIRKEGAARD, J. A., and DESMARCHELIER, J. M. 1995. Biofumigation: isothiocyanates released from *Brassica* roots inhibit growth of the take-all fungus. *Plant and Soil* 162:107–112.

BEST, K. F. and MCINTYRE, G. I. 1975. The biology of Canadian weeds. 9. *Thlaspi arvense* L. *Can. J. Plant Sci*. 55:279–292.

BETZ, J. M. and FOX, W. D. 1994. High-performance liquid chromatographic determination of glucosinolates in *Brassica v*egetables, pp. 181–196, *in* Food Phytochemicals I: Fruits and Vegetables. ACS Symposium Series. American Chemical Society, Washington, DC.

BOYDSTON, R. A. and HANG, A. 1995. Rapeseed (*Brassica napus*) green manure crop suppresses weeds in potato (*Solanum tuberosum*). *Weed Tech*. 9:669–675.

BOYDSTON, R. A. and VAUGHN, S. F. 2002. Alternative weed management systems control weeds in potato (*Solanum tuberosum*). *Weed Tech*. 16:23–28.

BROWN, P. D. and MORRA, M. J. 1995. Glucosinolate-containing plant tissues as bioherbicides. *J. Agric. Food. Chem*. 43:3070–3074.

BROWN, P. D. and MORRA, M. J. 1997. Control of soil-borne plant pests using glucosinolate-containing plants. *Advan. Agron.* 61:167–231.

BROWN, P. D., MORRA, M. J., MCCAFFREY, J. P., AULD, D. L., and WILLIAMS, L. III. 1991. Allelochemicals produced during glucosinolate degradation in soil. *J. Chem. Ecol.* 17:2021–2034.

CHEW, F. S. 1988. Biological effects of glucosinolates, pp. 155–181, *in* H. G. Cutler (ed.). Biologically Active Natural Products: Potential Use in Agriculture. American Chemical Society, Washington, DC.

COLE, R. A. 1976. Isothiocyanates, nitriles and thiocyanates as products of autolysis of glucosinolates in Cruciferae. *Phytochemistry* 15:759–762.

DAXENBICHLER, M. E., SPENCER, G. F., CARLSON, D. G., ROSE, G. B., BRINKER, A. M., and POWELL, R. G. 1991. Glucosinolate composition of seeds from 297 species of wild plants. *Phytochemistry* 30:2623–2638.

DONKIN, S. G., EITEMAN, M. A., and WILLIAMS, P. L. 1995. Toxicity of glucosinolates and their enzymatic decomposition products to *Caenorhabditis elegans*. *J. Nematol.* 27:258–262.

ELBERSON, L. R., MCCAFFREY, J. P., and TRIPEPI, R. R. 1997. Use of rapeseed meal to control black vine weevil larvae infesting potted rhododendron. *J. Environ. Hortic.* 15:73–176.

FENWICK, G. R., HEANEY, R. K., and MULLIN, W. J. 1983. Glucosinolates and their breakdown products in food and food plants. *Crit. Rev. Food. Sci. Nutr.* 18:123–201.

GROSSMAN, J. 1993. Brassica alternatives to herbicides and soil fumigants. *IPM Pract.* 15:1–10.

HOLM, L., DOLL, J., HOLM, E., PANCHO, J., and HERBERGER, J. 1997. World Weeds: Natural Histories and Distribution. John Wiley & Sons, New York.

KIRKEGAARD, J. A. and SARWAR, M. 1999. Glucosinolate profiles of Australian canola (*Brassica napus annua* L.) and Indian mustard (*Brassica juncea* L.) cultivars: implications for biofumigation. *Aust. J. Agric. Res.* 50:315–324.

LEWIS, J. A. and PAPAVIZAS, G. C. 1971. Effect of sulfur-containing volatile compounds and vapors from cabbage decomposition on Aphanomyces euteiches. *Phytopathology* 61:208–214.

LÜTHY, J. and BENN, M. H. 1977. Thiocyanate formation from glucosinolates: A study of the autolysis of allylglucosinolate in *Thlaspi arvense* L. seed flour extracts. *Can. J. Biochem.* 55:1028–1031.

MAYTON, H. S., OLIVIER, C., VAUGHN, S. F., and LORIA, R. 1996. Correlation of fungicidal activity of *Brassica* species with allyl isothiocyanate production in macerated leaf tissue. *Phytopathology* 86:267–271.

MCLAFFERTY, F. W. and STAUFFER, D. 1989. Directory of Mass Spectral Data. John Wiley & Sons, New York.

MITICH, L. W. 1996. Field pennycress (*Thlaspi arvense* L.)—the stinkweed. *Weed Tech.* 10:675–678.

MOJTAHEDI, H., SANTO, G. S., HANG, A., and WILSON, J. H. 1991. Suppression of root-knot nematode populations with selected rapeseed cultivars as green manure. *J. Nematol.* 23:170–174.

MOJTAHEDI, H., SANTO, G. S., WILSON, J. H., and HANG, A. 1993. Managing *Meloidogyne chitwoodi* on potato with rapeseed as green manure. *Plant Dis.* 77:42–46.

MUELCHEN, A. M., RAND, R. E., and PARKE, J. L. 1990. Evaluation of crucifer green manures for controlling Aphanomyces root rot of peas. *Plant Dis.* 74:651–654.

OLIVIER, C., VAUGHN, S. F., MIZUBUTI, E. S. G., and LORIA, R. 1998. Variation in allyl isothiocyanate production within *Brassica* species and correlation with fungicidal activity. *J. Chem. Ecol.* 25:2687–2701.

PAPAVIZAS, G. C. 1966. Suppression of Aphanomyces root rot of peas by cruciferous soil amendments. *Phytopathology* 56:1071–1075.

PAPAVIZAS, G. C. and LEWIS, J. A. 1971. Effect of amendments and fungicides on Aphanomyces root rot of peas. *Phytopathology* 61:215–220.

RAMIREZ-VILLAPUDUA, J. and MUNNECKE, D. E. 1988. Effect of solar heating and soil amendments of cruciferous residues on *Fusarium oxysporum* f. sp. *conglutinans* and other organisms. *Phytopathology* 78:289–295.

RASK, L., ANDREASSON, E., EKBOM, B., ERIKSSON, S., PONTOPPIDAN, B., and MEIJER, J. 2000. Myrosinase: gene family evolution and herbivore defense in Brassicaceae. *Plant. Mol. Biol.* 42:93–113.

ROLLINS, R. C. 1993. The Cruciferae of Continental North America. Stanford University Press, Stanford, California.

SARWAR, M., KIRKEGAARD, J. A., WONG, P. T. W., and DESMARCHELIER, J. M. 1998. Biofumigation potential of brassicas. III. *In vitro* toxicity of isothiocyanates to soil-borne fungal pathogens. *Plant and Soil* 201:103–112.

SHOFRAN, B. G., PURRINGTON, S. T., BREIDT, F., and FLEMING, H. P. 1998. Antimicrobial properties of sinigrin and its hydrolysis products. *J. Food Sci.* 63:621–624.

SLATER, G. P. 1992. Allyl thiocyanate-natural product or artifact in crucifer extracts? *Chromatographia* 34:461–467.

SPRINGETT, M. B. and ADAMS, J. B. 1989. Identification of 1-cyano-2,3-epithiopropane in volatiles of a model system from Brussels sprouts (*Brassica oleracea* L. var. *bullata* subvar. *gemmifera* DC). *J. Sci. Food Agric.* 46:211–219.

TSAO, R., PETERSON, C. J., and COATS, J. R. 2002. Glucosinolate breakdown products as insect fumigants and their effect on carbon dioxide emission of insects. *BMC Ecology* 2:5.

UDA, Y., KURATA, T., and ARAKAWA, N. 1986. Effects of pH and ferrous ion on the degradation of glucosinolates by myrosinase. *Agric. Biol. Chem.* 50:2735–2740.

VANETTEN, C. H. and TOOKEY, H. L. 1983. Glucosinolates, pp. 15–30, *in* M. Rechcigl (ed.). Naturally Occurring Food Toxicants. CRC Press, Boca Raton, Florida.

VAUGHN, S. F. 1999. Glucosinolates as natural pesticides, *in* H. G. Cutler and S. J. Cutler (eds.). Biologically Active Natural Products: Agrochemicals. CRC Press, Boca Raton, Florida.

VAUGHN, S. F. and BERHOW, M. A. 1999. Allelochemicals isolated from tissues of the invasive weed garlic mustard (*Alliaria petiolata*). *J. Chem. Ecol.* 25:2495–2504.

VAUGHN, S. F. and BOYDSTON, R. A. 1997. Volatile allelochemicals released by crucifer green manures. *J. Chem. Ecol.* 23:2107–2116.

VAUGHN, S. F., BOYDSTON, R. A., and MALLORY-SMITH, C. A. 1996. Isolation and identification of (3-methoxyphenyl)acetonitrile as a phytotoxin from meadowfoam (*Limnanthe alba*) seedmeal. *J. Chem. Ecol.* 22:1939–1949.

VAUGHN, S. F., SPENCER, G. F., and LORIA, R. 1993. Inhibition of *Helminthosporium* strains by natural isothiocyanates. *Am. Pot. J.* 70:852–853.

WALKER, J. T. 1996. Crambe and rapeseed meal as soil amendments: nematicidal potential and phytotoxic effects. *Crop. Prot.* 15:433–437.

WARTON, B., MATTHIESSEN, J. N., and SHACKLETON, M. A. 2001. Glucosinolate content and isothiocyanate evolution—two measures of the biofumigation potential of plants. *J. Agric. Food Chem.* 49:5244–5250.

WILLIAMS-WOODWARD, J. L., PFLEGER, F. L., FRITZ, V. A., and ALLMARAS, R. R. 1997. Green manures of oat, rape and sweet corn for reducing common root rot in pea (*Pisum sativum*) caused by *Aphanomyces euteiches*. *Plant Soil* 188:43–48.